

Attorney Docket No.: DC-0230
Inventors: Mulligan-Kehoe, Mary Jo
Serial No.: 10/686,428
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Amendments to the Specification:

Please replace Example 1, beginning at line 23 of page 60, with the following rewritten Example:

--The DNA encoding the truncated PAI-1 proteins was obtained by deleting the porcine PAI-1 gene (*poPAI-1*) (Bijnens, et al. (1997) *Thromb. Haemost.* 77:350-356; Accession number Y11347; SEQ ID NO:3). The selection of gene fragments was based on the *poPAI-1* sequences that correspond to the human PAI-1 gene (*huPAI-1*) (Bosma, et al. (1988) *J. Biol. Chem.* 263:9129-9141; Accession number J03764; SEQ ID NO:4) sequence reported to code for functional domains in human PAI-1 (Reilly and Hutzelmann (1992) *supra*). Each DNA fragment was isolated from porcine aortic endothelial cells by reverse transcribing RNA into cDNA. The cDNA was made double-stranded in a polymerase chain reaction (PCR) containing porcine PAI-1-specific primers. Primers for ~~rPAI-1₂₃~~ amplification are known, for example, Mulligan-Kehoe, et al. ~~((2001) *supra*)~~ amplification of the cDNA encoding rPAI-1₂₃, were 5'-primer, 5'-GGAATTC AAGGAGCTATGG-3' (SEQ ID NO:5) and 3'-primer, 5'-GCTCTAGATTTCCTGGTGATG-3' (SEQ ID NO:6). The resulting product corresponds to nucleotides 444-999 of *poPAI-1* and 238-793 of *huPAI-1*. Primers used to PCR amplify the cDNA into double-stranded DNA coding for rPAI-1_{Δ23} are 5' primer: 5'-GGAATTCATGGATGAGATCAGCACGG-3' (~~SEQ ID NO:3~~ SEQ ID NO:7) and 3' primer: 5'-GCTCTAGATTTCCTGGCTGATG-3' (~~SEQ ID NO:4~~ SEQ ID NO:8). The resulting product corresponds to nucleotides 471-999 of *poPAI-1* and 265-793 of *huPAI-1*. Likewise, primers used to PCR amplify the cDNA into double-stranded DNA coding for rPAI-1_{Hep23}

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are 5' primer: 5'-GGAATTCATGCAGTTCAAGATTGAGGAGAAGGGC-3' (~~SEQ ID NO:5~~ SEQ ID NO:9) and 3' primer: 5'-GCTCTAGATTTCCTGGCTGATG-3' (~~SEQ ID NO:6~~ SEQ ID NO:8). The resulting product corresponds to nucleotides 390-999 of *poPAI-1* and 184-793 of *huPAI-1*. Furthermore, primers used to PCR amplify the cDNA into double-stranded DNA coding for rPAI-1₂₄ are 5' primer: 5'-GGAATTCAAGGAGCTCATGG-3' (~~SEQ ID NO:7~~ SEQ ID NO:10) and 3' primer: 5'-GCTCTAGATCAAGGCTCCATCAC-3' (~~SEQ ID NO:8~~ SEQ ID NO:11). The resulting product corresponds to nucleotides 444-1346 of *poPAI-1* and 238-1162 of *huPAI-1*. Underlined nucleotides denote restriction enzyme recognition sequences. PCR conditions for amplification of all three genes are known to one skilled in the art, for example, Reilly and Hutzelmann ((1992) *supra*). The PCR-amplified rPAI-1 DNA fragments were double-digested with *EcoRI* and *XbaI* (Roche, Indianapolis, IN) to create overhangs. The restricted DNA was ligated into *Pichia pastoris* yeast shuttle vector pGAPZαA (INVITROGEN™, Carlsbad, CA). The TOP 10 strain of *Escherichia coli* was transformed by electroporation as described (Mulligan-Kehoe, et al. (2001) *supra*). Following an overnight incubation at 37°C, colonies were selected and grown in low salt LB broth for 5-7 hours at 37°C. The DNA from each colony was isolated using a miniprep kit (Qiagen, Inc. Valencia, CA) to identify a clone containing each gene insert. Positive isolates were identified by restriction enzyme digests and were verified by sequencing. Each recombinant protein was expressed in *P. pastoris* as described (Reilly and Hutzelmann (1992) *supra*) and purified by affinity chromatography. The sequences of rPAI-1₂₃, rPAI-1_{Δ23}, rPAI-1_{Hep23}, and rPAI-1₂₄ DNA matched the known sequence

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of the corresponding segment of porcine PAI-1 DNA. Each rPAI-1 protein corresponded to its expected molecular weight.--